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Cellular distribution of the nicotinic acetylcholine receptor α7 subunit in rat hippocampus

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ABSTRACT

The hippocampus is a region of the mammalian brain that has been extensively studied due to its role in many forms of memory. To better understand hippocampal function, significant attention has focused upon the cellular distribution of ligand-gated ion channels. Despite strong cholinergic innervation from the basal forebrain and a dense expression of nicotinic acetylchoine receptors (nAChRs), the cellular distribution of subunits forming these receptors has received little attention. We used organotypic hippocampal slice cultures (OHSCs) to study native α7 subunits, which, unlike other nAChR subunits, form a homomeric receptor. Cell-surface biotinylation, cross-linking of surface proteins, and sub-cellular fractionation all revealed a very limited presence of the subunit at the plasma membrane. In contrast, subunits of other receptors displayed significant surface expression. Notably, subunits in adult hippocampal tissue were distributed in a fashion similar to that observed in OHSCs. To monitor α7 subunits contained in functional nAChRs, a colourimetric assay using α-bungarotoxin (a specific α7 nAChR antagonist) was developed, and revealed a majority of binding at the cell surface. To change α7 subunit distribution, OHSCs were treated with compounds known to affect other ionotropic receptors—insulin, genistein, and elevated external K^+; however, neither subunit surface expression nor antagonist binding was affected. Our data reveal that hippocampal neurons possess a large internal population of α7 subunits under basal conditions, which persists during stimuli affecting tyrosine phosphorylation or neuronal activity. The nature of the internal pool of α7 subunits remains to be determined, but should have important implications for hippocampal activity.

1. Introduction

The hippocampus is a relatively small structure embedded in the temporal lobe, but is the focus of great interest because of a presumably integral role in memory formation (Scoville and Milner, 1957; Bird and Burgess, 2008). Cholinergic terminals arising from the medial septum and vertical limb of the diagonal band in the basal forebrain innervate the hippocampus (Semba, 2000), and extensive evidence suggests that these afferents influence learning and memory (Gold, 2003; McKay et al., 2007). Furthermore, the cognitive dysfunction characteristic of Alzheimer's disease is thought to be in part related to degeneration of cholinergic input to the hippocampus (Bourin et al., 2003). Consequently, understanding cholinergic physiology, and, in particular, how acetylcholine and its agonists act upon hippocampal neurons, is essential to understanding how the structure functions and contributes to memory.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated, cation channels formed by the pentameric assembly of various alpha (α2-10) and beta (β2-4) subunits (Dani and Bertrand, 2007; Gaimarri et al., 2007). Although the variety of nAChR subunits allows for a diverse number of combinations, two predominant types exist within the hippocampus: high nicotine affinity, α-bungarotoxin (BGT) insensitive receptors that generally contain α4β2 subunits, and low nicotine affinity, BGT sensitive receptors formed exclusively with α7 subunits (Orr-Urtreger et al., 1997; Drisdel and Green, 2000). The homomeric α7 nAChR is widely expressed at hippocampal pyramidal neurons (Dominguez

Abbreviations: ACSF, artificial cerebrospinal fluid; AHSP, acute hippocampal slice preparation; AMPAR, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; BGT, α-bungarotoxin; BS^3, bis(sulfosuccinimidyl) suberate; BSA, bovine serum albumin; BSS, balanced salt solution; DIV, days *in vitro*; DMSO, dimethyl sulfoxide; DS, dissecting solution; $GABA_AR$, γ-aminobutyric acid subtype A receptor; GS, genistein; HRP, horseradish peroxidase; INS, insulin; MLK, skim milk powder; nAChR, nicotinic acetylcholine receptor; ND, non-detergent; OHSC, organotypic hippocampal slice culture; PBS, phosphate buffered saline; PFA, paraformaldehyde; PVDF, polyvinylidene fluoride; RIPA, radioimmunoprecipitation assay; SA, streptavidin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; SF, serum-free; TBST, tris buffered saline plus tween-20; UADB, universal antibody diluting buffer; WH, whole homogenate.

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del Toro et al., 1994) and GABAergic interneurons (Alkondon and Albuquerque, 2004), and is situated both pre- and post-synaptically at many synapses in the hippocampus (Fabian-Fine et al., 2001). The high concentration of the α7 nAChR in the hippocampus, combined with its high permeability to Ca^{2+} (Fucile, 2004), are key reasons underlying why the receptor is believed to be important in both hippocampal function and disease (Broide and Leslie, 1999; Freedman et al., 2000; Dani and Bertrand, 2007).

A growing body of work suggests that neuronal activity in the hippocampus is greatly influenced by finely regulated processes that alter the surface density of various receptors (Kennedy and Ehlers, 2006). Changes in the cellular distribution of receptors can be influenced by a number of factors, such as the production of constituent subunits, the proper assembly of these subunits, the trafficking of assembled proteins to the plasma membrane, and recycling or degradation pathways. Most of the current knowledge surrounding receptor localization has been acquired due to the significant attention directed toward ionotropic glutamate and GABA receptor subunits (Collingridge et al., 2004). Despite a functional profile and pattern of expression that indicates a widespread relevance to neural activity, the cellular distribution of native α7 nAChR subunits has received comparatively little attention. Given the strong likelihood that the α7 subunit plays a role in both hippocampal function and disease, we used models of this structure to study the subunit's cellular location under basal conditions and following stimuli that have been shown to induce the movement of other ion-channel receptor proteins.

2. Experimental procedures

2.1. Organotypic hippocampal slice culture preparation

Organotypic hippocampal slice cultures (OHSCs) were prepared according to methods described previously (Mielke et al., 2005, Fig. 1). For each culture, 6–8 Sprague–Dawley rat pups at post-natal day 8 were, in succession and according to procedures approved by the institutional animal care committee, anaesthetized with ketamine (1 mg/pup; Bioniche, Belleville, ON, Canada), and placed on ice for 2 min. After decapitation, brains were rapidly removed, and hippocampi dissected over ice. A McIlwain tissue chopper (Mickle Laboratory Engineering Co., Surrey, U.K.) was used to prepare 400 μm transverse slices, which were then cooled in dissecting solution (DS) consisting of Gey's BSS (Sigma, Oakville, ON, Canada; all subsequent reagents were from Sigma unless otherwise noted) supplemented with 35 mM glucose. Slices were then examined under a dissecting microscope, and those considered suitable (generally 10–12 per animal) allowed to recover for 60–90 min at 4 °C in DS prior to being transferred to sterile, semi-porous Millicell-CM inserts (3–4 slices/insert; Millipore, Bedford, MA, USA) placed in six well-cultured trays (Falcon, VWR, Mont-Royal, PQ, Canada) containing 1 mL medium/well. Slice medium consisted of 50% MEM with HEPES modification, 25% Hank's BSS, 25% heat inactivated horse serum, 1 mM glutamine, and 35 mM glucose (305–315 mOsm). Slices were maintained in a 5% CO_2 (balance air) humidified incubator at 36 °C. Complete media changes were done 1 day after plating and 3×/week thereafter.

To examine the developmental expression of proteins, seven slices were pooled from each culture on the day of preparation, and 24 slices from the same culture were pooled after 7, 14, 21, and 28 days *in vitro* (DIV). Cultured slices were washed 2× with cooled PBS, removed from membranes, and transferred to 400 μL of non-ionizing lysis buffer [10 mM Tris, 25 mM EDTA, 100 mM NaCl, 1% (v/v) Triton X-100, 1% (v/v) NP-40, pH 7.40], supplemented with a protease inhibitor cocktail. Following manual homogenization (12 complete strokes with a Teflon pestle in a 2 mL glass tube) over ice, lysates were centrifuged at 1000 × *g* for 10 min (4 °C), and supernatant protein concentration determined with a BioRad DC protein assay kit.

2.2. Whole hippocampus and acute hippocampal slice preparation

Female Sprague–Dawley rats, 200–300 g, were anaesthetized with halothane and decapitated in accordance with procedures approved by the institutional animal care committee. Brains were rapidly removed (~60 s) and immediately placed in cooled (1–4 °C) artificial cerebrospinal fluid (ACSF) containing (in mM): 124.0 NaCl, 3.0 KCl, 1.2 NaH_2PO_4, 1.0 $MgSO_4$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, 10.0 glucose, 10.0 HEPES, aerated with carbogen (95% O_2 and 5% CO_2), pH 7.37–7.43, 300–310 mOsm. Hippocampi were removed and either homogenized for differential centrifugation, or oriented in the same direction on the platform of a McIlwain tissue chopper (Fig. 1B). Beginning approximately 10 mm from the septal poles, 350 μm thick slices were cut and placed in turn upon fine mesh inserts that were rested within an interface incubation chamber (3–4 slices per platform) wherein the ACSF was continuously gassed with carbogen. The incubation chamber was kept at 34.5 ± 0.5 °C, and at least 60 min allowed prior to beginning experiments.

2.3. Cell-surface protein biotinylation

OHSCs (20–26 DIV) were placed over ice and washed twice with ACSF (1–4 °C). Immediately prior to each experiment, the biotinylation solution was prepared by diluting biotin (Pierce Biotechnology, Rockford, IL, USA) in ACSF (0.5 mg/mL). Each

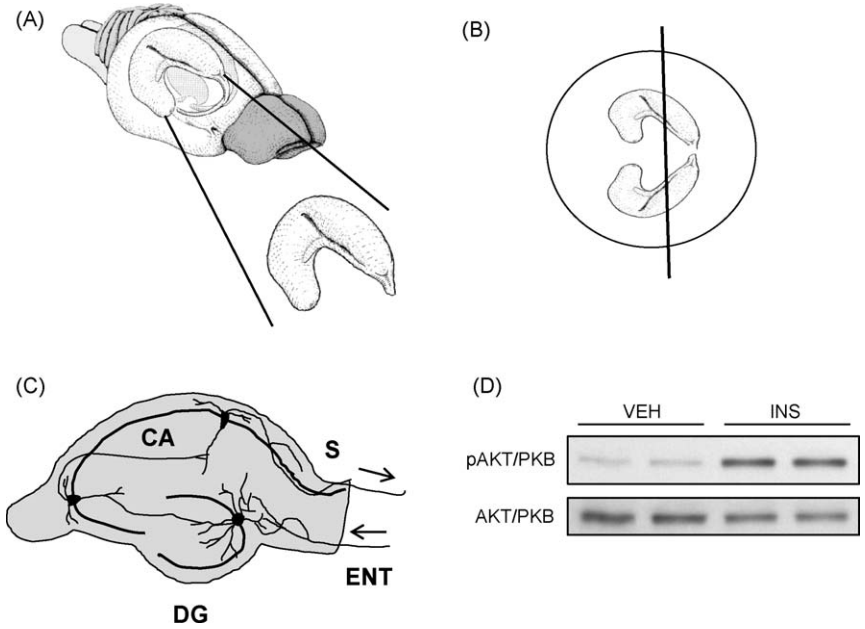

Fig. 1. The hippocampal slice preparation. (A) Situated within the telencephalon, the hippocampal formation is a curved, cylindrical structure that can be easily dissected from rodent brain. (B) To prepare hippocampal slices, isolated hippocampi are placed upon a platform, and then thinly sectioned at an angle approximately perpendicular to the longitudinal axis of the structure. (C) A representative tissue slice displaying the four highly connected regions that comprise the hippocampal formation: the entorhinal cortex (ENT), the hippocampus proper (CA subfields or *cornu ammonis*), the dentate gyrus (DG), and the subiculum (S). Arrows indicate the general direction of hippocampal afferents and efferents. (D) Organotypic hippocampal slice cultures (OHSCs) can be maintained for several weeks, and are responsive to physiological stimuli. The images present homogenates from OHSCs at 19 DIV that were treated for 15 min with either vehicle (VEH; serum-free culture medium) or 100 nM insulin (INS), and then immunoblotted for evoked phosphorylation of AKT/PKB, which is a principle downstream component of the insulin signaling cascade.